Monday, August 1, 2016 (Christine Tang, Kevin Chen)

Objective: Gibson assemble invasin parts. **(Christine Tang)**

1. Followed procedure on Benchling.
    a. Calculated DNA mix using Duesseldorf inSilico calculator

pFd007.Tet backbone	100 ng
Invasin part 1	33.2 ng
Invasin part 2	28.8 ng
Invasin part 3	30.3 ng
Invasin part 4	24.8 ng

    b. Used 5 microliters of DNA total with 15 microliters of isothermal ligase buffer.
    c. Incubated 50 degrees Celsius for 15-60 minutes
    d. Zymocleaned
    e. Transformed into XL1EC cells
    f. Plated on LB/CM(34)

Conclusion: Colony PCR and grow cultures tomorrow.

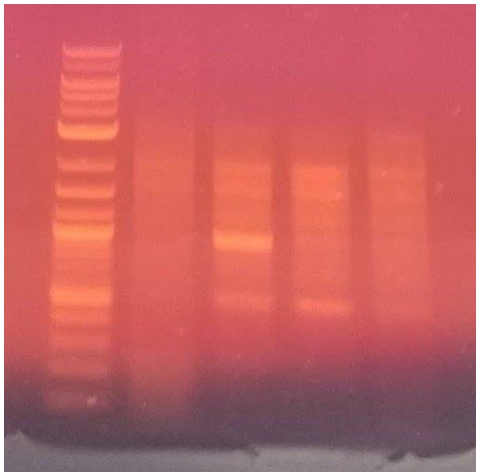
Objective: To colony PCR pTet670. **(Kevin Chen)**

1. Transformed pPCB6 into XL1EC.
2. Plated on LB/Spec(100).
3. Incubated 16 h at 37 degrees Celsius.

Tuesday, August 2, 2016 (Christine Tang, Margaret Lie)

Objective: To send pTet-iRFP670 for sequencing

1. Ran gel for pTet samples 1-4. Sample 2 looked the best.



2. Miniprepped all four samples. Discarded samples 1 and 4 because concentrations were too low.
3. Sent samples pTet670 2 and 3 to Genewiz for sequencing. Used primers iGEM 15 and 63.

Conclusion: Concentrations were low ut still sent samples 2 and 3 for sequencing. Will check results when they come in.

Objective: to grow cultures for constitutive iRFP713 (Christine Tang)

1. Made 2 cultures for constitutive iRFP713.
2. Put in 37 degrees Celsius for 16-18 hours overnight.

Conclusion: 1 culture will be miniprepped tomorrow. The other culture will go into glycerol stock.

Objective: to colony PCR invasin. (Christine Tang)

1. Followed Colony PCR protocol on Benchling. Made 4 samples. Used primers iGEM gibson 1 and iGEM gibson 7. Made 4 cultures and incubated at 37 degrees Celsius for 18 hours.
2. Ran gel on 0.8% agarose.

Conclusion: There should be a band at 3.2 kb for all four samples. None of them have bands at 3.2 kb. Will still miniprep and send for sequencing tomorrow.

Objectives: to PCR *fdhf* promoter from MG1655. To amplify *fdhf* for insertion into pSB1C3. **(Margaret Lie)**

1. Set up PCR reaction.

    a.

Material	Volume
dH2O	37 µL
Thermopol Buffer	5 µL
dNTPs	1 µL
iGEM 67	2.5 µL
iGEM 68	2.5 µL
MG1655	1 µL
VentR Polymerase	1 µL

Thermocycling:

Temp. (°C)	Time	Cycle
95	5:00	
95	0:30	
60	0:30	25-35X
72	0:08	
72	10:00	
4	∞	

Wednesday, August 3, 2016 (Kevin Chen, Christine Tang)

Objective: To sequence invasin from August 2, 2016.

1. Sent invasin samples to Genewiz for sequencing with primers iGEM 33, gibson 10, gibson 3 and gibson 1.
    a. iGEM 33 - for part 4
    b. iGEM gibson 10 - for part 3
    c. iGEM gibson 3 - for part 2
    d. iGEM gibson 1 - for part 1
    e. Used 4 sequencing primers because each invasin part is big.

Conclusion: Will check sequencing results when they come in. If they are good, we will save the samples for the mammalian cell culture.

Objective: to make pTET-iRFP670 cultures **(Christine Tang)**

1. Picked parts of the streak from a colony PCR plate Kevin Chen made from August 1, 2016. Grew them in 3 mL LB/Kan.
2. Grew cultures for 16-18 hours at 37 degrees Celsius.

Conclusion: Will miniprep 1 culture for mammalian cell, send another for re-sequencing and store the third in glycerol stock.

Thursday, August 4, 2016 (Christine Tang)

Objective: to send one culture of pTet-iRFP670 for re-sequencing, save one for mammalian cell work and to put one in glycerol stock.

1. Made glycerol stock of pTet670 #3.
2. Miniprepped and nanodropped the other two cultures.
    a. pTet670 #1 - 86.0 ng/µL
    b. pTet670 #2 - 70.9 ng/µL
3. Saved pTet670 #1 for mammalian cell work. Sent #2 for resequencing.

Conclusion: Will check sequencing results when they come in.

Tuesday, August 9, 2016 (Christine Tang)

Objective: to check sequencing results for invasin.

1. For invasin - only three samples passed quality control. All of these results were bad.

Conclusion: For invasin, all sequencing results are 99%-100% identical to a ferrodoxin gene petF, which is a part in pFd007.Tet backbone that should have been cut out during PCR before Gibson assembly. Will redo PCR of pFd007.Tet backbone tomorrow.

Wednesday, August 10, 2016 (Christine Tang)

Objective: to redo PCR of pFd007.Tet template for the purpose of trying to create invasin.

1. Set up PCR reaction:

a.

Material	Volume	Concentration
dH2O	32.4 µL	-
5x Phusian HF Buffer	10 µL	-
dNTPs (50x)	1 µL	10 mM
iGEM gibson 2	2.5 µL	10 µM
iGEM gibson 8	2.5 µL	10 µM
pFd007.Tet template	1.1 µL (1 ng)	10 µM
Phusian DNA polymerase	0.5 µL	100x stock

Thermocycling:

Temperature (Celsius)	Time	Cycle
98	0:30	
98	0:10	
62	0:30	35x
72	1:26	
72	10:00	
4	∞	

2. Ran a 0.8% agarose gel at 110 V.

Conclusion: The gel did not look good (should have been a band at 2.8 kb). Will try touchdown PCR next time.

Monday, August 26, 2016 (Kevin Chen, Margaret Lie)

1. Transformed pBAD/HisB-iRFP 670, pBAD/HisB-iRFP 713, pETM6 - E12 - VioABECD in *E. Rosetta*
2. Plated on ampicillin plates. Incubated at 37 degrees Celsius.
3. Grew liters of cultures of violacein and harvested.

Tuesday, August 27, 2016 (Margaret Lie)

1. Transformed pBAD/HisB-iRFP 670 and 713 in *E. coli* with heme oxygenase (pSR34 Bvd).
2. Plated.

Wednesday, August 28, 2016 (Margaret Lie)

1. Grew liquid cultures of pBAD/HisB-iRFP670 and 713 in *Bvd Ros*.

Friday, August 30, 2016 (Margaret Lie)

Objective: to colony PCR *fdhf*

1. Used Vent polymerase to colony PCR *fdhf* using primers VF, VR. Elongation temperature set to 51.6 degrees Celsius. Annealing time set to 13 seconds for 1 cycle.